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Research Article

Effects of Extruded Soy Protein on the Quality of Chinese Steamed Bread

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Five different extruded soy protein isolates (ESPIs) were obtained by extrusion and denoted by IVD1, IVD2, IVD3, IVD4, and IVD5. Then the SDS-PAGE results showed that the subunits of SPI decreased after extrusion, especially the subunits of 90.8, 32.8, and 31.3 kDa, whereas no isopeptide bond was formed. Although SPI improved both the development time (DT) and stability (S) of dough, ESPIs increased S but the DT decreased from 4.3 min to 1.8–2.0 min. Texture profile analysis (TPA) results showed that the hardness and chewiness of Chinese steamed bread (CSB) decreased in the order wheat flour+IVD2 (WF+IVD2), WF+SPI, WF+IVD4, WF+IVD1, WF+IVD3, WF, and WF+IVD5. As regards color, the total color ΔE decreased except for the WF+IVD1 (56.22); its positive and negative trends of L^* and b^* were invariant with the SPI or ESPIs mixture, whereas a^* showed a positive trend. The sensory score increased from 82.7 to 83.4 with 3% of SPI addition and up to 87.8 when the substitution was IVD1. Therefore, SPI treated by extrusion may significantly improve the quality of CSB.

1. Introduction

Chinese steamed bread (CSB), a traditional fermented principal food of China, is gaining widely consumed by people in China and the emigrated Chinese people of many other Southeastern Asian countries [1]. CSB is mainly composed of wheat flour (WF), water, and yeast, accounting for about 40% of wheat consumption for breakfast item in China every year [2]. WF is the major ingredient of steamed bread and comprises mainly starch (about 70%–75%), water (about 14%), and proteins (about 10%–12%) [3, 4]. The quantities of protein and starch are important factors determining the gluten strength of dough; that is, CSB quality depends on dough strength. With the lack of lysine for WF, the amino acid balance of CSB was poor. What is worse, furosine is an indirect measurement of α-N-formyl-(ε-N-deoxyglucose)-Lys, which is the major component of blocked Lys present after the early Maillard reaction [5]. Bread may come into being a part of furosine when adding bran, rye, or maize to the wheat flour, respectively. Nevertheless a synergistic effect of suppressing furosine formation with some soybean flour mixture may occur simultaneously [6].

Soybean protein contains eight essential amino acids, especially Lys. It is mainly composed of 7S globulin or β-conglycinin and 11S globulin or glycinin. β-conglycinin is a trimer comprising three subunits (α', α, and β), whereas glycinin comprises six subunits, each making up an acidic polypeptide and a basic polypeptide [7]. Soybean protein isolate (SPI) is widely used in the food industry because of its favorable water-holding capacity, oil-binding capacities, and other functional properties [8, 9], and many researchers have also discovered the functions of soybean protein in alleviating osteoporosis and inhibiting hyperlipidemia and other physiological health functions [10, 11]. Therefore, adding some soybean protein to WF can not only improve the nutritional value of CSB but also be beneficial to human health.

WF mixed with some soy protein flour can provide high protein content and improve the balance of amino acids; it could also reduce the rate at which frozen storage attenuates quality of dough [12]. Bread utilizing heat-treated (steamed and roasted) soy flour was likely to have a less beany odour and taste than those with non-heat-treated (raw and germinated) soy flour. Nevertheless, the bread with heat-treated flour is perceived to have a better appearance and

loaf volume than that with non-heat-treated flour [13]. The sensory scores of bread increased from 40.2 to 52.2, when mixing WF with 6% of soy protein, and it does not have a negative effect on the body's absorption of iron, calcium, zinc, and other minerals [14].

Though oxidising improvers and surfactant improved dough strength of soy-wheat bread, they could not weaken soy-wheat bread beany flavors, especially at the high contents [15]. Furthermore, the bioavailability of soy protein is limited by the trypsin inhibitors and hemagglutinin. These problems may be resolved by mild extrusion [16]. Extrusion technology is widely used in industrialization because it has a large production capacity, high utilization of raw materials, product diversification, energy-conservation feature, and other advantages [17]. Meanwhile, soy protein can endure complicated changes with the condition of high temperature, pressure, and shear forces in the extruder [16], whereas it has not been reported that extruded soy protein isolates (ESPIs) were applied to CSB.

The present work aimed to improve the *in vitro* digestibility of SPI and achieve large-scale production of CSB using the technology of extruded soy protein, which was explored with a DS32II double-screw extruder. The rheological properties and microstructure of dough were evaluated by farinograph and scanning electron microscope (SEM) analyses, and then CSB quality was assessed by sensory evaluation, colorimetry, and texture profile analysis (TPA). The ability of ESPIs to improve CSB properties, which can have significance in people's daily lives, was determined.

2. Materials and Methods

2.1. Materials. SPI (moisture 6.8% and protein 92.4%) provided by Henan Kunhua Biological Technology Co., Ltd., Special number 1 flour (moisture 13.7%, protein 11.1%, and ash 4.8%) was purchased from Zhengzhou Jinyuan Industry Co., Ltd. All reagents and chemicals were of analytical grade, unless otherwise specified.

2.2. Preparation of ESPIs. About 1000 g of SPI was mixed with 400 g of water by using a milling dough maker until no large boulders exist. Then appropriate feeding speed, discharging speed, and extrusion temperature were selected, to extrude SPI using a DS32II double-screw extruder (Jinan Saixin Machinery Co., Ltd.), as shown in Table 1.

Table 1: The technological conditions of ESPIs.

ESPIs	Feeding speed (r/min)	Discharging speed (r/min)	Extrusion temperature/°C
IVD1	50	50	120
IVD2	50	50	120
IVD3	75	63	110
IVD4	50	63	110
IVD5	63	57	110

IVD1, IVD2, IVD3, IVD4, and IVD5 denoted as five different samples of ESPIs.

2.3. In Vitro Digestibility (IVD). *In vitro* digestibility of protein was determined using trypsin (250 U/mg; trypsin 1 : 250) and pepsin (250 U/mg protein; obtained from porcine gastric mucosa) enzyme system according to the reported method [18] with micromodification. About 1.0 g of protein was suspended in 20 mL of 0.1 M HCl and mixed with 0.1 g of pepsin in 1 mL of 0.01 M HCl. The mixture was incubated with slight shake at 37°C, for 6 h, and then added to 10 mL water; 5 mL of 0.50 M NaOH and 10 mL of 0.10 M phosphate buffer (pH 8.0) containing 20 mg trypsin were added. The digested mixture was slightly shaken for 12 h at 37°C and then 5 mL of aqueous solution of 20% (w/w) 5-sulfosalicylic acid dihydrate was added. Precipitated proteins were removed by filtration, and then the nitrogen content of supernatant was determined by the Kjeldahl nitrogen analysis. *In vitro* digestibility of protein was expressed as percentage of enzymatic digestion, as per the following formula:

$$\text{Enzymatic digestion\%} = \frac{\text{Nitrogen (insoluble protein) released by enzyme}}{\text{Total nitrogen content of sample}} \times 100\%. \quad (1)$$

2.4. SDS-PAGE. SDS-PAGE was applied to a discontinuous buffer system according to the slightly modified method of Tang et al. [19] using 12% separating gel and 5% stacking gel. The protein (20 μg/mL, SPI or ESPIs mixture mixed with sample buffer, 1 : 1 v/v) was electrophoresed after heating for 4 min in boiling water. Every sample (10 μL) was added to each lane. Before the sample exited the stacking gel, electrophoresis was performed at 18 mA and the other was at 35 mA. The gel was dyed with 0.1% Coomassie brilliant blue (R-250) in 25% ethanol and 8% acetic acid (ethanol : acetic acid : water, 250 : 80 : 670 v/v/v) and then destained in 25% ethanol and 8% acetic acid.

2.5. Dough Rheological Properties. Dough rheological properties were determined with a farinograph (Brabender, Duisburg, Germany) according to the American Association of Cereal Chemists standard method 54-21 using about 300 g of composite flour containing 3% protein. Parameters, conducted at the average of double measurements, such as water absorption (WAC), dough development time (DT), stability time (*S*), degree of softening (DS), and farinograph quality number (FQN) were acquired from the software to evaluate the dough rheological properties.

2.6. Scanning Electron Microscopy (SEM) Analysis of Dough. Before drying to the critical point, freeze-dried dough was broken into approximately 0.5 cm thick piece spot adjuncts and then coated with gold particles for 110 s. Manual smoothening of the fracture surface was difficult, so we acquired a relatively smooth area to observe the cross section of dough using SEM (JSM-6490LV, JEOL, Japan) analysis at magnifications of 900.

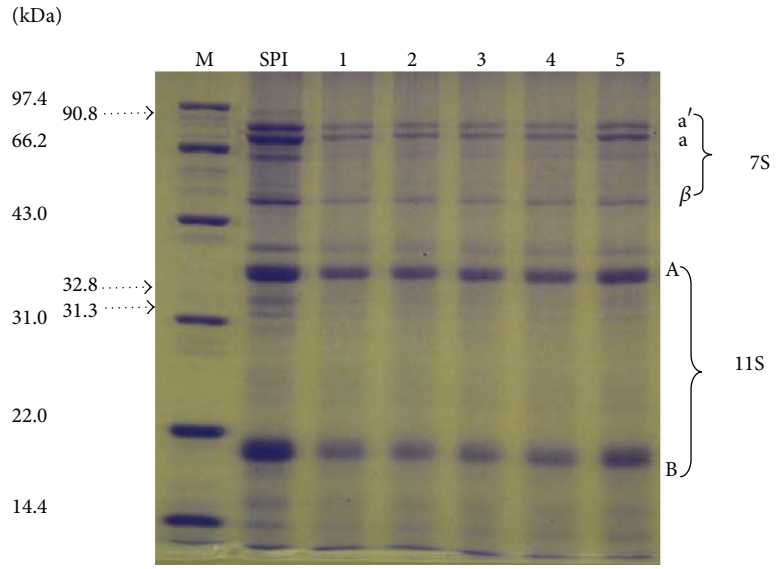

Figure 1: The SDS-PAGE patterns of SPI and ESPIs. M-protein markers: (1) SPI, (2) IVD1, (3) IVD2, (4) IVD3, (5) IVD4, and (6) IVD5.

2.7. Preparation of CSB. CSB was prepared according to the reported method [1, 2] with slight modifications using composite flour (220 g), dry yeast (1.7 g), and water (78% of farinograph water absorption). After mixing for 4.0 min at low speed and then kneading to form dough, the dough was sheeted seven times and divided into three pieces (100 g per piece). These pieces were rounded and molded manually and then fermented for 45 min in a fermentation room at 38°C and 80% relative humidity. The fermented dough was steamed for 23 min using a steam cooker and boiling water.

2.8. Assessment of CSB Quality. CSB sensory scores were evaluated by a ten-person evaluation panel according to the method reported by [20, 21] with slight modifications. CSB score includes specific volume (weighting, 20), exterior appearance (20), skin color (10), interior structure (15), taste (20), and flavor (20). CSB was cut into 15 mm thick slices with a bread knife for texture profile analysis (TPA) performed by a TA-XI2i PLUS Texture Analyzer (Stable Micro Systems, Ltd., Surrey, England) with the Pasta Firmness/Stickiness Rig probe (P36R). The test parameters were as follows: pretest speed, 5 mm/s; test speed, 1 mm/s; posttest speed, 1 mm/s; and compression, 50%. After TPA analysis, the color of CSB was tested by colorimetry which was based on a system that is very closely related to the perception of color difference to a human observer for most objects; that is, L^* (0 indicates black and 100 indicates white), a^* ($+a^*$ indicates redness and $-a^*$ indicates greenness), b^* ($+b^*$ indicates yellowness and $-b^*$ indicates blueness), and ΔE represented the total color of substance [22]. Texture and color measurement of CSB were the average of three measurements at the same conditions:

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\right]^{1/2}. \quad (2)$$

2.9. Statistical Analysis. The analysis of variance (one-way ANOVA) was performed to analyze all measurements. Significant differences among the treatment group were analyzed by Duncan's test with SPSS software (Version 16.0, SPSS Inc., Chicago, IL, USA) and graphics were generated with Origin 8.5 software.

3. Results and Discussion

3.1. Characterization of SPI and EPSI. After extrusion and drying for 30 h at 60°C, ESPIs and SPI digestibility were measured. With more action sites of protease exposed for SPI after extrusion, its digestibility had significantly increased. The digestibility of SPI and five different ESPIs (denoted as IVD1, IVD2, IVD3, IVD4, and IVD5) was as follows: 82.2%, 84.3%, 85.9%, 88.3%, 91.1%, and 92.4% respectively, and their SDS-PAGE patterns are shown in Figure 1.

The subunits of soy 7S (α', α, and β) and 11S (A and B) globulin and other subunits were all decreased, especially the subunits of 90.8 kDa, 32.8 kDa, and 31.3 kDa, whereas no isopeptide bond was formed. Extrusion processing exposed some hydrophobic group for SPI and resulted in complex changes with regard to the hydrogen bonds, disulfide bond, and hydrophobic interaction and their interactions were weakened, which may decrease protein solubility and parts of subunits content [23, 24]. Thus, the IVD of ESPIs improved, but no obvious variations in subunits occurred.

3.2. Properties of Dough. Results of the composite dough farinograph indicated that the water absorption (WAC), dough stability (*S*), and farinograph quality number (FON) of dough were increased but the degree of softening (DS) decreased when mixed with 3% of SPI or ESPIs. Meanwhile, dough development time (DT) decreased in the order WF+SPI (5.60 min), WF (4.30 min), WF+IVD1 (2.00 min), WF+IVD2 (1.85 min), WF+IVD4 (1.85 min), WF+IVD3 (1.80 min), and WF+IVD5 (1.70 min). WAC increased and then decreased with ESPIs digestibility increase, whereas WAC ranged within WF+SPI (60.85%) and WF (58.85%). Although the *S* of WF+SPI (6.70 min), WF+IVD1 (6.60 min),

Table 2: Effects of SPI and ESPIs on farinographic properties of dough.

Samples	WAC/%	DT/min	S/min	DS/FE	FQN
WF	58.85 ± 0.04^{a}	4.30 ± 0.00^{b}	4.55 ± 0.31^{a}	89.00 ± 5.37^{c}	62.50 ± 4.02^{a}
WF+SPI	60.85 ± 0.04^{d}	5.60 ± 0.09^{c}	6.70 ± 0.27^{b}	78.50 ± 1.34^{cb}	79.50 ± 1.34^{d}
WF+IVD1	59.60 ± 0.09^{b}	2.00 ± 0.27^{a}	6.60 ± 0.36^{b}	67.00 ± 3.58^{ab}	73.00 ± 0.89^{bc}
WF+IVD2	60.40 ± 0.00^{c}	1.85 ± 0.13^{a}	6.55 ± 0.04^{b}	64.00 ± 1.79^{a}	76.50 ± 1.34^{cd}
WF+IVD3	60.35 ± 0.04^{c}	1.80 ± 0.00^{a}	7.00 ± 0.09^{b}	64.50 ± 1.34^{a}	79.00 ± 0.89^{d}
WF+IVD4	59.75 ± 0.13^{b}	1.85 ± 0.31^{a}	6.30 ± 0.09^{b}	70.50 ± 3.13^{ab}	68.50 ± 2.24^{b}
WF+IVD5	59.35 ± 0.13^{b}	1.70 ± 0.18^{a}	6.75 ± 0.31^{b}	65.50 ± 3.13^{a}	73.50 ± 0.45^{c}

WF+SPI, WF+IVD1, WF+IVD2, WF+IVD3, WF+IVD4, and WF+IVD5, denoted as the dough or CSB containing 3% of SPI, IVD1, IVD2, IVD3, IVD4, and IVD5. WAC: water absorption, DT: development time, *S*: dough stability, DS: degree of softening (ICC/12 min after max), and FQN: farinograph quality number. Values within a column with different letters are significantly different ($P < 0.05$).

Table 3: Effect of SPI and ESPIs on the color of Chinese steamed bread.

Samples	L^*	a^*	b^*	ΔE
WF	82.73 ± 0.63^{c}	-0.53 ± 0.03^{a}	19.16 ± 0.12^{a}	56.12 ± 0.56^{bc}
WF+SPI	82.41 ± 0.19^{bc}	0.17 ± 0.01^{d}	19.93 ± 0.03^{b}	56.09 ± 0.18^{bc}
WF+IVD1	82.78 ± 0.26^{c}	-0.10 ± 0.00^{b}	19.35 ± 0.13^{a}	56.22 ± 0.22^{c}
WF+IVD2	80.99 ± 0.07^{a}	0.46 ± 0.01^{e}	20.63 ± 0.04^{d}	55.03 ± 0.06^{a}
WF+IVD3	81.98 ± 0.20^{bc}	0.13 ± 0.00^{c}	19.96 ± 0.05^{c}	55.69 ± 0.20^{abc}
WF+IVD4	81.61 ± 0.17^{ab}	0.15 ± 0.03^{cd}	20.25 ± 0.29^{c}	55.46 ± 0.05^{ab}
WF+IVD5	81.63 ± 0.42^{ab}	0.12 ± 0.01^{c}	20.85 ± 0.08^{d}	55.71 ± 0.42^{abc}

Values within a column with different letters are significantly different ($P < 0.05$).

WF+IVD2 (6.55 min), WF+IVD3 (7.00 min), WF+IVD4 (6.30 min), and WF+IVD5 (6.75 min) did not significantly differ, the DT decreased obviously as ESPIs were added. The reason may be that soy protein was amphiphilic, and extrusion caused the partially hydrophobic group to be exposed and promoted the gluten cross-linking and disulfide bond forming [16, 23, 24]. Both SPI and ESPIs could improve the rheological properties of dough; above all ESPIs may save the cost of mixture time in the food industry (Table 2).

SEM was used to observe the gluten network structure and distribution of starch granules. Dough had a continuity of gluten network structure and formed a closer gluten network structure than WF, when WF was mixed with 3% of SPI or ESPIs. The results showed that starch granules were embedded in the gluten network structure and they were basic and correlated with farinograph finding (Figure 2). The reason may be that SPI or ESPIs increased the S-S bonds of gluten network and contributed to the gluten protein cross-linking [16, 24]. The extrusion process enhanced gluten network tightness strength due to the denaturation that involved the intramolecular hydrogen bonds, van der Waals forces, weakened hydrophobic interaction, and exposed hydrophobic group and thiol [16, 23]. Thus, SPI treated by extrusion may significantly improve the rheological properties of dough.

3.3. Texture of CSB. Results of TPA showed that CSB hardness and chewiness were decreased in the order WF+IVD2 (3564.60 g/2741.42 g), WF+SPI (3277.48 g/2626.27 g), WF+IVD4 (3218.20 g/2464.96 g), WF+IVD1 (3141.07 g/2396.21 g), WF+IVD3 (2992.88 g/2334.40 g), WF (2841.68 g/2229.66 g), and WF+IVD5 (2773.03 g/2183.33 g). This result showed that SPI extruding can improve the tenacity of CSB in general, though there was no significant difference with regard to WF, WF+IVD1, WF+IVD3, and WF+IVD5. Similar to our data, bread utilizing soy protein isolate (SPI) may increase hardness and chewiness due to dilution of the gluten matrix, interchange of disulphide bonds between gluten proteins and SPI, and increasing the dough viscosity as the absorption of water increased by SPI [13, 25, 26]. As the TPA of CSB was significantly associated with the sensory evaluation, particularly the hardness and chewiness had significant influence on its comprehensive score [27]. High quality CSB should have an appropriate hardness and chewiness; being too large or too small may be bad for the quality (Figure 3).

3.4. Color of CSB. With addition of 3% of SPI or ESPIs, red and yellow values increased and brightness decreased except for the WF+IVD1. Moreover, the L^* of WF (82.73), WF+IVD1 (82.78), WF+IVD3 (81.98), and WF+SPI (82.41) did not have significant difference, and the L^* value of CSB decreased compared with WF, except for WF+IVD1. Values of a^* indicated that color transformed from greenness to redness when 3% of SPI, IVD2, IVD3, IVD4, or IVD5 was added. Although there were no significant differences in b^* for WF compared with WF+SPI or WF+ESPIs except for WF+IVD1, the color was still yellow. ΔE did not have significant difference when it came to WF, WF+SPI, WF+IVD1, WF+IVD3, and WF+IVD5. It may be attributed to the browning by polyphenol oxidase in processing and other natural pigments in WF with different colors such as B vitamins, flavonoid compounds, and carotenoid etcetera [28, 29]. Moreover, isoflavones and other color substances of SPI also have effect on CSB color [30, 31]. However, with addition of 3% of ESPIs, ΔE did not have significant difference which may be due to the fact that the color substances and structure of SPI were destroyed after extrusion (Table 3) [23, 24].

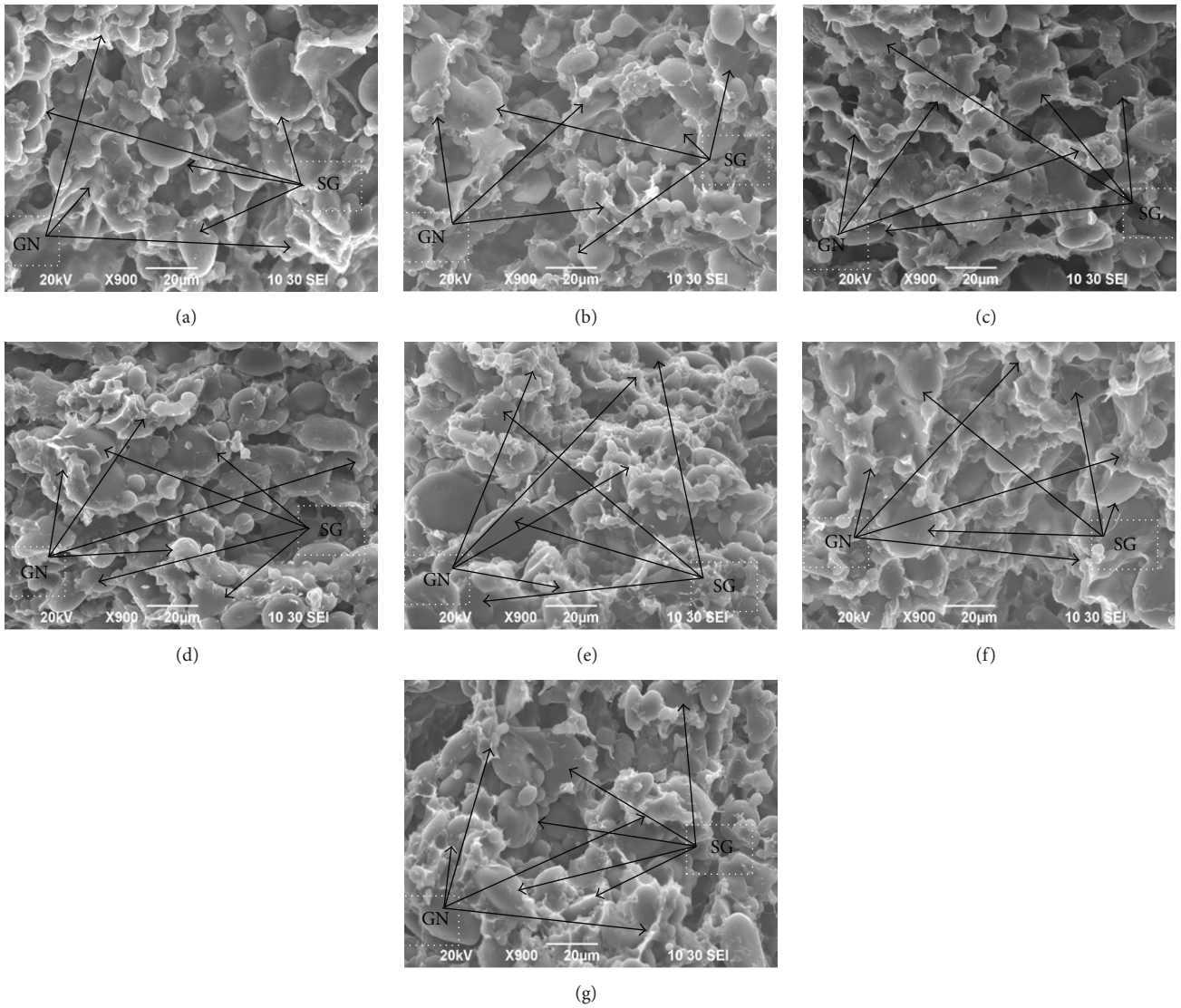

Figure 2: Effects of SPI and ESPIs on microstructure of dough. Samples of (a), (b), (c), (d), (e), (f), (g), GN, and SG represented the dough of WF, WF+SPI, WF+IVD1, WF+IVD2, WF+IVD3, WF+IVD4, WF+IVD5, gluten networks, and starch granules.

Table 4: Effect of SPI and ESPIs on the sensory score of Chinese steamed bread.

Samples	Specific volume	Exterior appearance	Skin color	Interior structure	Taste	Flavor	Total score
WF	13.7 ± 0.4^{a}	17.2 ± 0.7^{c}	8.4 ± 0.5^{b}	11.6 ± 1.0^{ab}	15.8 ± 0.7^{a}	16.0 ± 1.0^{a}	82.7 ± 1.8^{a}
WF+SPI	14.0 ± 0.6^{a}	17.6 ± 1.8^{c}	6.0 ± 0.6^{a}	11.8 ± 1.1^{abc}	16.6 ± 0.5^{ab}	17.4 ± 0.5^{a}	83.4 ± 2.0^{a}
WF+IVD1	15.0 ± 0.0^{b}	18.0 ± 0.6^{c}	8.2 ± 1.1^{b}	13.2 ± 0.7^{c}	16.6 ± 0.8^{ab}	16.4 ± 1.0^{a}	87.8 ± 1.1^{b}
WF+IVD2	15.0 ± 0.0^{b}	18.0 ± 1.0^{c}	7.8 ± 0.4^{b}	10.4 ± 1.0^{a}	17.0 ± 0.9^{ab}	16.2 ± 1.1^{a}	84.4 ± 2.1^{ab}
WF+IVD3	15.0 ± 0.0^{b}	17.0 ± 0.9^{bc}	8.6 ± 0.8^{b}	12.0 ± 0.6^{bc}	17.2 ± 0.7^{ab}	17.6 ± 1.0^{a}	87.4 ± 1.0^{b}
WF+IVD4	15.0 ± 0.0^{b}	15.4 ± 1.3^{ab}	7.6 ± 0.5^{b}	11.8 ± 1.1^{abc}	17.6 ± 1.0^{b}	16.2 ± 1.5^{a}	83.6 ± 2.7^{a}
WF+IVD5	15.0 ± 0.0^{b}	13.8 ± 0.9^{a}	6.4 ± 0.5^{a}	13.2 ± 0.7^{c}	17.2 ± 1.1^{ab}	16.8 ± 0.9^{a}	82.4 ± 1.4^{a}

Values within a column with different letters are significantly different ($P < 0.05$).

3.5. Sensory Assessment of CSB Quality. The sensory scores and cross section of CSB were summarized in Table 4 and Figure 4, respectively. Specific volume and interior structure significantly affect CSB quality, and they were all almost improved in comparison with the control as 3% of SPI or ESPIs were used, especially WF+IVD1 and WF+IVD3. In terms of exterior appearance, no significant difference was observed when involving WF+SPI (17.6 scores), WF+IVD1 (18.0), WF+IVD2 (18.0), and WF+IVD3 (17.0) compared with the control. The sensory scores and cross section of CSB with regard to the WF+IVD1 (87.8 scores), WF+IVD2 (84.4), and WF+IVD3 (87.4) had higher sensory scores and were more

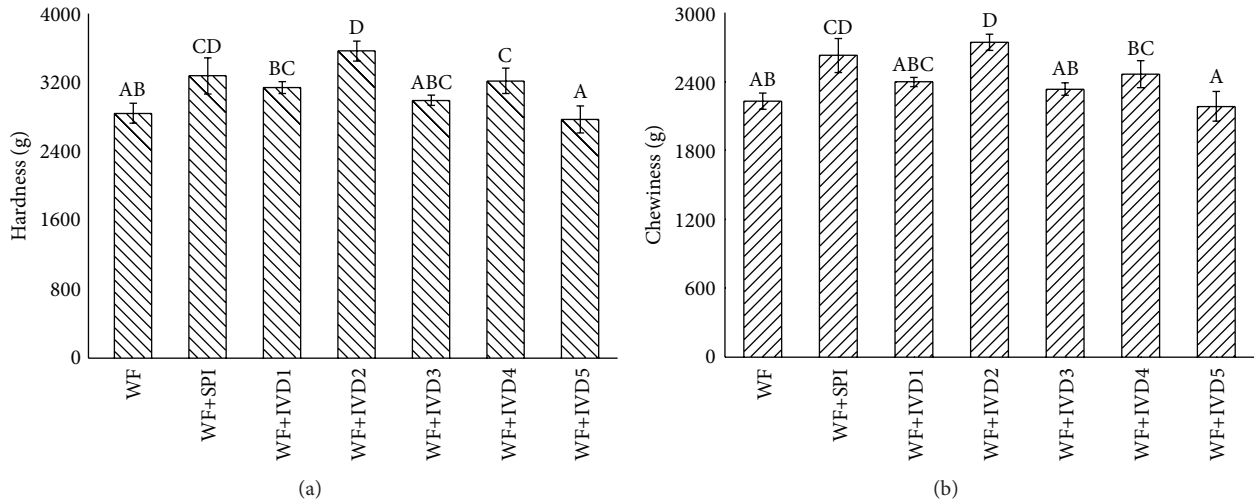

Figure 3: Influence of SPI and ESPIs on hardness (a), chewiness (b), and springiness of CSB. Values within a column with different letters are significantly different ($P < 0.05$).

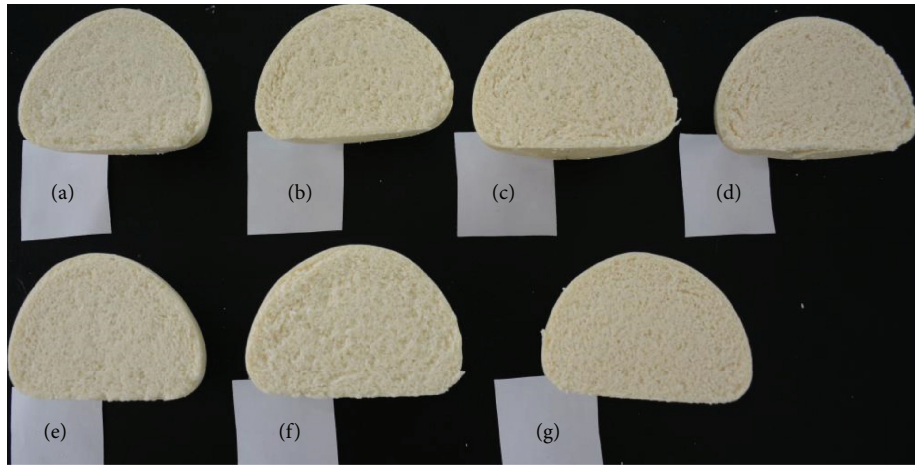

Figure 4: Effects of SPI and ESPIs on the cross section of CSB samples of (a), (b), (c), (d), (e), (f), and (g) representative of the CSB of WF, WF+SPI, WF+IVD1, WF+IVD2, WF+IVD3, WF+IVD4, and WF+IVD5.

exquisite, implying that CSB with appropriate hardness and chewiness, closer control group color, and exquisite cross section had a better sensory property. The reason may be that SPI and ESPIs could form a greater polymer network with the gluten protein and act as the nitrogen source of yeast and they could also increase the S-S bonds of gluten network [24, 28], thereby improving the specific volume and interior structure of CSB. Moreover, the slight savoury released by SPI or ESPIs after cooking may improve the taste and flavor of CSB, especially by weakening the beany flavor by extrusion process. In the study by Shin et al. [13], similar to our data, the bread with heat-treated (steamed and roasted) soy flour has a lower beany odour and taste than those with non-heat-tread (raw and germinated) soy flour.

4. Conclusions

After extrusion, the IVD of ESPIs improved while the subunits of soy 7S (α', α, and β) and 11S (A and B) globulin decreased, especially the subunits of 90.8 kDa, 32.8 kDa, and 31.3 kDa. Although SPI and ESPIs both improved the rheological properties of dough, ESPIs may save the cost of kneading time in the food industry. Compared to WF and WF+SPI CSB, the total score of WF+IVD1, WF+IVD2, and WF+IVD3 CSB had the higher scores and exquisite cross section. It demonstrated that SPI treated by extrusion may significantly improve the nutrition and quality of CSB.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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